Algal H_2-Production Systems: Creation of Designer Alga for Efficient and Robust Production of H_2

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Objectives

Develop advanced renewable photolytic hydrogen generation technologies through creation of a designer alga by genetic insertion of a proton channel into the photosynthetic thylakoid membrane. By 2015, demonstrate an engineering-scale biological system that produces hydrogen at a plant-gate cost of $10/kg projected to commercial scale.

Technical Barriers

This project addresses the following technical barrier from the Hydrogen Production section of the Hydrogen, Fuel Cells and Infrastructure Technologies Program Multi-Year R,D&D Plan:

- J. Rate of Hydrogen Production

Approach

Creation of designer alga for efficient and robust production of H_2 through genetic insertion of a proton channel into the thylakoid membrane.

Accomplishments

This is a new project. Because of the Congress and DOE budget situation under the "Continuing Resolution," this project did not receive any funding until April 2003. In April 2003, the DOE authorized $50K for J. Lee (1) to attend the Merit Review and (2) to make preparation for a full start of the project on October 1, 2003. So, the following are the results of our preliminary studies and the project preparation work.

- Identified the technical variables (problems) needed to optimize algal H_2 production.
- Designed a method—genetic insertion of a proton channel into the thylakoid membrane—to solve the identified technical problems.
- Demonstrated the proof of principle through preliminary studies.

Future Directions

Since this is a new project that is yet to be fully started, its proposed milestones represent the future directions. If funding support is fully provided, the project objective could be achieved within 4-5 years with the following milestones.

- Year 1—Design and construction of DNA sequence coding for polypeptide proton channel

- Year 2—Genetic transfer of hydrogenase promoter-linked polypeptide proton-channel DNA into algal strain DS521
- Year 3—Characterization and optimization of the polypeptide proton-channel gene expression
- Year 4—Demonstration of efficient and robust production of H_2 in designer alga (ready for next phase: scale-up and commercialization)

Introduction

This R&D project will create algal H_2-production systems by a new and novel approach that has recently been developed at ORNL. In this approach, a "designer alga" for efficient and robust H_2 production will be created by genetic insertion of hydrogenase promoter-programmed polypeptide-proton channels into photosynthetic thylakoid membranes.

Approach

We have recently developed a systematic approach to create a "super" photosynthetic organism—a "designer alga" that is specifically designed for production of molecular hydrogen through photosynthetic water splitting (ORNL Invention Disclosure ID 0981).[1] This designer alga will be able to overcome the four major physiological problems that currently challenge researchers and investors in the field of photosynthetic H_2 production: (1) restriction of photosynthetic H_2 production by accumulation of a proton gradient, (2) competitive inhibition of photosynthetic H_2 production by CO_2, (3) requirement of bicarbonate binding at photosystem II (PSII) for efficient photosynthetic activity, and (4) newly discovered O_2 sensitivity in algal H_2 production.

The key element of our proposed approach is creation of a designer alga for efficient and robust production of H_2 through genetic insertion of a programmable polypeptide proton channel into the thylakoid membrane. The genetic insertion of programmable thylakoid-membrane proton channels is proposed to be achieved by transformation of a host alga with a genetic vector that contains a polypeptide proton-channel gene linked with a hydrogenase promoter. The envisioned super alga that can be created by the proposed work should be able to perform autotrophic photosynthesis using ambient-air CO_2 as the carbon source and grow normally under aerobic conditions such as in an open pond. When the algal culture is grown and ready for H_2 production, the proton-channel gene will then be expressed simultaneously with the induction of the hydrogenase enzyme under anaerobic conditions because of the use of the hydrogenase promoter. The expression of the proton-channel gene should produce polypeptide proton channels in the thylakoid membrane, thus dissipating the proton gradient across the thylakoid membrane without adenosine triphosphate (ATP) formation.

As illustrated in Figure 1, our recent experimental studies with the proton uncoupler carbonyl cyanide m-chlorophenylhydrazone (FCCP) have already demonstrated that insertion of a proton-conductive channel in the thylakoid membrane could significantly enhance H_2 production by eliminating the problems of both the proton-gradient accumulation and the newly discovered alternative O_2 sensitivity that is dependent on the proton gradient.[2] Furthermore, the cessation of photophosphorylation (ATP formation) caused by action of the proton channels can, in turn, switch off the Calvin cycle activity (CO_2 fixation), which requires ATP and competes with the ferredoxin (Fd)/hydrogenase H_2-production pathway for the photosynthetically generated electrons. As a result, the competitive inhibition of H_2 production by CO_2 will now be eliminated, and photosynthetic H_2 production in the designer alga will be able to occur in the presence of CO_2. Since photosynthetic H_2 production in a successful designer alga no longer requires a CO_2 (HCO_3^-)-free environment, the requirement for HCO_3^- binding at photosystem II (PSII) for efficient photosynthetic activity will also no longer be a problem. The requirement can now be

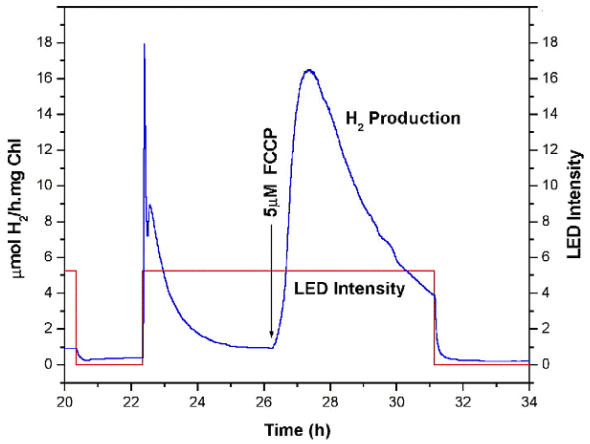

Figure 1. Stimulation of photosynthetic H_2 production in C. reinhardtii 137c following addition of the proton uncoupler FCCP in a background atmosphere of 1000-ppm O_2. Addition of 5 μM FCCP produced a dramatic increase in H_2 production, followed by a slow decay. The slow decay is due to a side effect of FCCP, known as ADRY,[3] in which FCCP gradually inhibits PSII activity. This ORNL experimental result indicates that use of a polypeptide proton channel that does not have the ADRY effect could enhance H_2 production by eliminating the problems of both the proton-gradient accumulation and the newly discovered alternative O_2 sensitivity.

satisfied by leaving some CO_2 in the medium. In conclusion, the use of polypeptide proton channels in the thylakoid membrane can provide four advantages for H_2 photoevolution: (1) the accumulation of a proton gradient that impedes the photosynthetic electron transport from water to Fd/hydrogenase will be prevented; (2) the competitive inhibition of photosynthetic H_2 production by CO_2 (Calvin cycle activity) will be eliminated; (3) the requirement for bicarbonate binding at PSII for efficient photosynthetic activity will be satisfied; and (4) the newly discovered O_2-sensitive pathway that competes with the H_2-production pathway for photosynthetically generated electrons could also be avoided by the dissipation of the proton gradient with the switchable proton channel.

Results

The proof-of-principle experimental results from our preliminary studies were also presented in a poster presentation at the May 2003 DOE Merit Review Meeting. We are now making preparations for a full start of this project on October 1, 2003. Because of the current funding limit, the technical lab work of this project will not be able to start until the arrival of the FY 2004 funding support.

Conclusions

Creation of designer alga by genetic insertion of a proton channel into the thylakoid membrane is one of the key R&D tasks that are required for the photobiological H_2-production system to work. We have already developed a systematic approach to achieve the proposed work. The proof of principle for this designer alga H_2-production R&D project has been demonstrated through preliminary studies.

References

1. Lee, J. W., and E. Greenbaum 2001. "Method for creating efficient and robust photosynthetic H_2-production systems," *ORNL Invention Disclosure* ID 0981.

2. Lee, J. W., and E. Greenbaum 2002. "A new oxygen sensitivity in photosynthetic H_2 production," *Applied Biochemistry and Biotechnology*, vol. 105-108, pp. 303-313.

3. Samuilov, V. D., E. L. Barsky, and A. V. Kitashov 1995. "ADRY agent-induced cyclic and non-cyclic electron transfer around photosystem II," *Photosynthesis: from Light to Biosphere*, P. Mathis (ed.), Vol. II, 267–270. The Netherlands, Kluwer Academic Publishers.

FY 2003 Publications/Presentations

1. Lee, J. W. and E. Greenbaum 2003 "A new oxygen sensitivity and its potential application in photosynthetic H_2 production," *Applied Biochemistry and Biotechnology*, vol. 105-108, pp. 303-313.

2. Lee, J. W., L. Mets, and E. Greenbaum 2002 "Improvement of photosynthetic CO_2 fixation at high light intensity through reduction of chlorophyll antenna size," *Applied Biochemistry and Biotechnology*, vol. 98-100, pp. 37-47.

3. Lee, J. W. 2003 "Overcoming nation's roadblocks to photosynthetic H_2 production," presented at the National Hydrogen Association's 14th Annual Meeting, March 4-6, 2003, Washington, DC.

Special Recognitions & Awards/Patents Issued

1. Lee, J. W. and E. Greenbaum 2001 "Method for creating efficient and robust photosynthetic H_2-production systems," ORNL Invention Disclosure ID 0981; U.S. Patent Application filed.